JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., APRIL 17, 1916 NO. 3

DETERMINATION OF STEARIC ACID IN BUTTER FAT[1]

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INTRODUCTION

Oils and fats are composed largely of neutral glyceryl esters together with small amounts of free fatty acids and unsaponifiable matter. Formerly the esters were considered simple glycerids, compounds of glycerol and three radicals of the same fatty acids. At present the opposite view seems to prevail and mixed glycerids are said to predominate in most products. The subject is controversial and difficult of solution. The constituents would be the same, however, in either case, whether combined as simple or complex molecules. The object of a technical examination of oils and fats is to isolate, identify, and determine the various fatty acids, glycerol, and unsaponifiable bodies, although, as Lewkowitsch asserts, this is not attainable in the present state of our knowledge. Certain progress has been made in determining different constituents of fats by indirect methods, such as iodin absorption, acetyl number, and molecular-weight calculations. Direct methods of fractional distillation, crystallization, and solubility of various salts have not, as a rule, proved sufficiently discriminative for quantitative use.

Fatty acids constitute about 95 per cent of most oils and fats and characterize the products to a large extent. The necessity of accurate methods for the quantitative determination of these acids has long been recognized not only from the standpoint of pure science but especially in physiological studies having as the object the measurement of the effect of different food groups on the production of body and milk fats. Many methods have been proposed since the publication of the work of Chevreul nearly 100 years ago, but few, if any, have met with general approval. After several years' investigations of the Partheil and Ferie method (7),[2] which proved unsatisfactory in the authors'[3] hands, a study of methods for determining stearic acid in butter fat was undertaken.

[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number to "Literature cited," p. 113.

[3] Mr. Reed was associated with the senior author in the earlier stages of the work and Mr. Buckley in the later.

EARLIER INVESTIGATIONS

For the separation of stearic from other fatty acids, David (1) recommended a special alcohol and dilute acetic-acid solution saturated with stearic acid at 15° C., in which solution oleic acid was shown to be soluble.

The Hehner and Mitchell (3) method for isolating stearic from other fatty acids was based on the hypothesis that a mixture of fatty acids heated with a solvent saturated at a given temperature with the acid under determination might be expected on cooling to that temperature to crystallize the whole of the acid sought, provided the other constituents did not increase the solubility. The solvent employed was methylated alcohol (94.4 per cent) saturated with stearic acid at 0.2° C., prepared by chilling a solution of 3 gm. to 1 liter overnight in ice water and siphoning off the saturated mother liquor through a small thistle tube covered with fine calico, using suction. The tests were conducted in a similar manner, taking from 0.5 to 5 gm. of insoluble acids (according to content) to 100 c. c. of alcohol-stearic-acid solution. Shaking was found to increase precipitation. Supersaturation and esterification were recognized as possible sources of error. The method gave concordant results with solid fats containing considerable stearic acid, but slight, if any, precipitate from the acids of butter fat and from mixtures of the acids of Japan wax and pure stearic acid.

Emerson (2) noted considerable variation in the content of different saturated solutions and found that supersaturation seemed to occur when less than 0.7 gm. to 100 c. c. was employed in preparing the solution. The formation of ethyl ester appeared to be a source of error and to have increased the apparent solubility of the stearic acid.

Kreis and Hafner (5) showed that small amounts of stearic acid below 0.1 gm. to 100 c. c. of a saturated solution formed supersaturated solutions, and that less than 0.05 gm. gave low and extremely variable results, even upon the addition of crystals of stearic acid.

Lewkowitsch (6, p. 556–559) claimed that the method yielded capricious results with mixtures of stearic, palmitic, and oleic acids, and that in many cases the results were entirely unreliable when other acids were present. He stated that a considerable proportion of lauric acid would prevent the complete precipitation of stearic acid, even when supersaturated alcohol-stearic-acid solutions were used, and that acids of higher melting point, when present, such as arachic, behenic, etc., would appear in the separated acids. He reported a precipitate of 0.49 per cent from butter fat, of which a portion might be arachic and myristic acids.

The results obtained by various investigators indicate that the solubility of stearic acid increases with the strength of the alcohol, but the figures reported are too variable to warrant further deductions (Table I).

TABLE I.—*Solubility of stearic acid, according to various investigators*

Investigator.	Approximate strength of alcohol.	Stearic acid to 100 c. c.	Saturation of 100 c. c. at 0° C.
	Per cent.	*Gm.*	*Gm.*
Hehner and Mitchell (3, p. 323)	94.4	0.2 to 0.5	0.1400 to 0.1580
Emerson (2, p. 1754)	95.5	.7	.1223
Do	95.1	.7	.1139
Do	94.5	.7	.1035
Kreis and Hafner (5)	95	.5	.1220 to .1310
Lewkowitsch (6, p. 164)	94.4	.3	.0814
Do	94.4	.7	.0810 to .1082
Ruttan (8, p. 440)	100	.....	.373

PRELIMINARY WORK

In view of what has been stated, the outlook for another investigation was not promising, although Lewkowitsch's final arraignment of the process was not published until nearly a year after the work was undertaken. The subject was of sufficient importance, however, to warrant additional study whatever the outcome.

APPARATUS.—To insure a uniform temperature for crystallization, a tank was constructed of ⅞-inch lumber (20 inches long, 10 inches wide, and 20 inches deep), lined with galvanized iron, provided with a tight cover, and raised by legs to a convenient working height. For icing, a basket (13½ by 6 by 18 inches) of galvanized screening of $\frac{5}{16}$-inch mesh, holding probably 30 pounds of broken ice, was found very satisfactory. The insulation of wood, together with the large volume of water and ice, proved inadequate to meet the requirements of the case, and it was necessary to install in one corner of the tank a pump run by a motor, to keep the water in continuous circulation. With this apparatus a constant temperature of about 0.1° C. was easily maintained (fig. 1, 2).

Several factors had to be considered in the selection of containers in which the tests were to be conducted. They must be of a form, size, and weight suitable for weighing the charge on analytical balances, easily held in position in the tank, and such that the alcoholic solution could be removed while still in the tank, leaving the crystalline residue. After numerous experiments with globe-shaped separatory funnels and filtering tubes, 8-ounce sterilizer bottles were adopted and have been found fairly satisfactory. The bottles are of narrow cylindrical form (2 by 6¾ inches) and are held in place in the tank by pockets of wire screening, with only the rubber stopper and a small portion of the neck projecting out of the water. The solution is siphoned off by means of a small thistle tube (¼-inch bulb) having a felt of absorbent cotton weighing 0.020 gm. supported by a glass bead and covered with a piece of batiste.

REAGENTS.—For the preparation of an alcohol-stearic-acid solution constituents of high quality were deemed essential for satisfactory work. The purification of alcohol had been a subject for study for a number of years in connection with the ordinary analysis of oils and fats, and excellent results were finally secured by treatment with silver nitrate and caustic lime and redistillation. A strength of 95.25 per cent proved a satisfactory solvent for fatty acids, and greater strength was not considered necessary or even advisable.

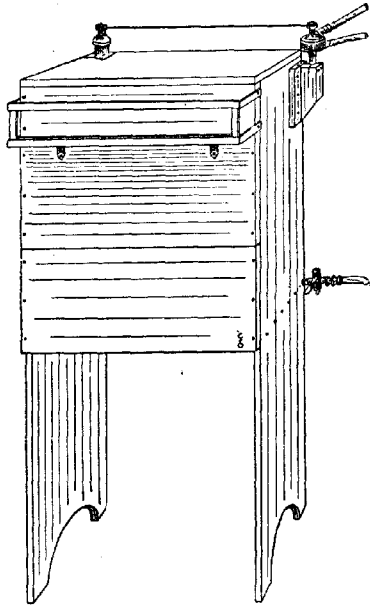

FIG. 1.—Exterior of constant-temperature crystallization tank.

One lot of stearic acid, a mixture of several grades, was purified by fractional distillation of the ethyl ester in vacuo and subsequent repeated crystallization of the separated acids from alcohol as previously described (4). Another lot of acid with a molecular weight of 271.13 was purified by 10 or more crystallizations from alcohol to a molecular weight of 284.25, and a second portion to 284.71, although the resulting leaflets were less perfect than those obtained by the former process.

When using separatory funnels and filtering tubes, alcohol-stearic-acid solutions, saturated at 0.1° C., applied to the insoluble acids of butter at the rate of 150 c. c. to 0.5 gm. of material, seldom yielded an appreciable amount of precipitate on standing, even with the addition of crystals of stearic acid and thorough agitation. Solutions testing about 0.22 and 0.24 gm. of stearic acid to 150 c. c. gave somewhat higher results, although of erratic and untrustworthy character. In the attempt to develop a method with this apparatus, over 140 determinations were made on butter acids, stearic acid, mixtures of butter and stearic acids, stearic and oleic acids, and stearic, myristic, and oleic acids. The object was not attained, and most of the data will be omitted, as

they would serve no useful purpose, merely indicating the time and labor involved. The results, however, with solutions of stearic acid appear to warrant certain deductions.

Solutions containing from 0.25 to 0.29 gm. of stearic acid to 150 c. c. crystallized, leaving a mother liquor of unlike composition (saturation).

The saturation varied inversely with the quantity of stearic acid present.

Presumably, therefore, supersaturation occurred as a result of insufficient stearic acid (Table II).

The time of standing may have had some influence, but when in excess of 24 hours it was of minor consequence.

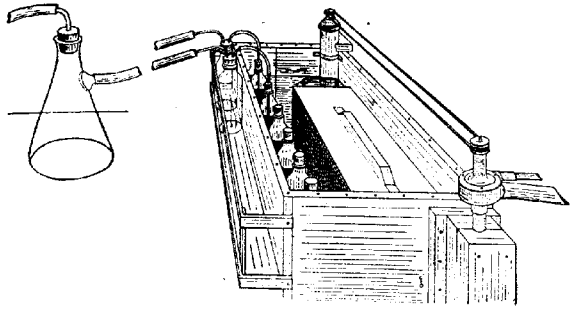

FIG. 2.—Interior of constant-temperature crystallization tank.

The form of the container as viewed in the light of subsequent work was a factor of some importance; a globe-shaped vessel was less effective than a narrow, cylindrical one of large surface.

TABLE II.—*Crystallization of stearic acid from solutions of different content, using separatory funnels*

Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).
	Gm.	*Gm.*			*Gm.*	*Gm.*	
0.2406	0.0100	0.0130	0.1584	0.2400	0.0304	0.0640	0.1376
.2406	.0150	.0254	.1535	.2400	.0354	.0733	.1347
.2406	.0150	.0315	.1494	.2400	.0475	.0872	.1335
.2406	.0400	.0859	.1298	.2400	.0481	.0910	.1314
.2406	.0450	.0995	.1241	.2400	.0491	.0910	.1321
.2400	.0200	.0426	.1449	.2400	.0498	.0960	.1292
.2400	.0251	.0544	.1405				

Stearic-acid solutions were found to crystallize more readily and with greater uniformity in sterilizer bottles than in separatory funnels, probably owing to the more rapid chilling of the narrow column of liquid and more thorough filtration.

Table III shows the amount of stearic acid crystallized from solutions of different content and the saturation of the mother liquor.

TABLE III.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

Alcohol.	Stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).	Alcohol.	Stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).
C. c.	*Gm.*	*Gm.*		*C. c.*	*Gm.*	*Gm.*	
150	0. 2000	0. 0000	.....	150	0. 3670	0. 1880	0. 1193
150	. 2400	. 0020	0. 1587	150	. 3800	. 2000	. 1200
150	. 2705	. 0485	. 1480	150	. 4000	. 2210	. 1193
150	. 2815	. 0700	. 1410	150	. 4080	. 2260	. 1213
150	. 3055	. 1110	. 1297	150	. 4200	. 2435	. 1177
150	. 3215	. 1280	. 1290	150	. 4650	. 2980	. 1113
150	. 3475	. 1680	. 1197	150	. 5000	. 3255	. 1163
150	. 3600	. 1815	. 1190	150	. 6000	. 4315	. 1123

TABLE IV.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

Alcohol-stearic-acid solution (0.3990 gm. in 150 c. c.).	Alcohol.	Equivalent in stearic acid (grams in 150 c. c.).	Precipitate.	Saturation (grams in 100 c. c.).
	C. c.		*Gm.*	
100	50	0. 2660	0. 0555	0. 1403
110	40	. 2926	. 0980	. 1297
120	30	. 3192	. 1500	. 1128
130	20	. 3458	. 1745	. 1142
140	10	. 3724	. 2055	. 1113
150	0	. 3990	. 2335	. 1103

APPLICATION OF CRYSTALLIZATION METHOD

The facility with which alcohol-stearic-acid solutions crystallize increased with the concentration. Solutions of 0.40 to 0.45 gm. to 150 c. c. formed crystals readily, gave a satisfactory amount of precipitate, and when applied to the insoluble acids of butter yielded an additional amount from that source. This would indicate that if the stearic-acid content of the solution is sufficient, crystallization of stearic from butter acids is no more difficult than from other products. The results were very concordant for a crystallization method when all details of manipulation were strictly observed: The water maintained at the required level, properly iced at all times, and the pump run continuously at good speed. A gentle agitation of the solution after standing overnight in the ice tank assisted in completing the precipitation, but anything in

the nature of shaking reduced the fragile crystals to a mass and rendered filtration extremely difficult or impossible.

EXPERIMENTAL METHOD IN DETAIL

Five-tenths of a gram of melted insoluble acids are placed in an 8-ounce sterilizer bottle and 150 c. c. of an alcohol-stearic-acid solution (3 gm. to 1,000 c. c.), accurately measured with a pipette at 30° C., added. The bottle is sealed with a solid-rubber stopper, shaken at a gradually increasing temperature until a clear solution is obtained, placed immediately in a pocket of the ice tank, and allowed to stand overnight. The following morning the solution is gently agitated by inverting the bottle several times, and in the afternoon it is siphoned off as thoroughly as possible by means of a small thistle tube and a perforated rubber stopper, using suction. The residue is dissolved in ethyl ether, transferred to a tared 140 c. c. wide-mouth Erlenmeyer flask, the ether carefully distilled off, the residue dried at 100° C., and weighed. As saturation may vary somewhat with the amount of stearic acid present and as the quantity of solution retained by the precipitate depends in a measure on the amount of precipitate, blanks are run on a weight of stearic acid equivalent to that expected in the test. By deducting the additional stearic acid taken from the weight recovered the true blank for the alcohol-stearic-acid solution is obtained.

NATURE OF THE PRECIPITATE

To ascertain whether the crystalline substance obtained from butter acids was stearic acid or a mixture, the residues from a number of tests (one being insufficient for accurate work) were combined and the molecular weight determined by saponification. Such a determination made after securing satisfactory control of the stearic-acid method gave 284.64, theoretically 284.288. The melting point was not determined, as it was considered less reliable than the molecular weight.

INFLUENCE OF DIFFERENT FATTY ACIDS ON PRECIPITATION OF STEARIC ACID

Numerous tests were made in an effort to determine whether lauric, myristic, palmitic, and oleic acids had any effect on the crystallization of stearic acid and, if so, the nature and extent of such action. Table V will serve to illustrate.

According to molecular-weight determinations the lauric and palmitic acids were of excellent quality and the myristic and oleic acids somewhat inferior.

Lauric, myristic, and oleic acids in relatively large amounts showed no appreciable influence on the crystallization of stearic acid. Palmitic acid, on the other hand, noticeably increased the solubility and affected the crystalline structure of the precipitate.

TABLE V.—*Effect of different fatty acids on precipitation of stearic acid*

Alcohol-stearic acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Other acids taken.	Precipitate.	Saturation (grams in 100 c. c.).
	Gm.	*Gm.*	*Gm.*	
STEARIC ACID				
0.3990	0.1000	.....	0.3420	0.1047
.3990	.1015	.....	.3430	.1050
.3990	.1035	.....	.3415	.1073
.3990	.1000	.....	.3405	.1057
LAURIC ACID				
0.3990	.1030	0.4000	.3455	.1043
.3990	.1000	.4000	.3415	.1050
.3990	.1000	.4000	.3430	.1040
.3990	.1010	.4000	.3450	.1033
MYRISTIC ACID				
0.3990	.1000	.4000	.3495	.0997
.3990	.1010	.4000	.3480	.1013
.3990	.1000	.4000	.3490	.1000
.3990	.1000	.4000	.3515	.0983
PALMITIC ACID				
0.3990	.1055	.4000	.3135	.1273
.3990	.1000	.4030	.2980	.1340
.3990	.1010	.2500	.2965	.1357
.3990	.1040	.2500	.3065	.1310
.3990	.1050	.2000	.3085	.1303
OLEIC ACID				
0.3990	.1070	.4220	.3515	.1030
.3990	.1010	.4255	.3440	.1040
.3990	.1000	.4000	.3485	.1003
.3990	.1035	.4000	.3460	.1043

The addition of palmitic acid to butter acids reduced the amount of stearic acid recovered in the test. Some of our more recent determinations indicated that the solvent action of palmitic acid can be counteracted in a large measure, if not entirely, by increasing the relative amount of stearic acid in solution. With butter acids of average palmitic acid content, an alcohol-stearic-acid solution, containing at least 3 gm. of stearic acid to the liter, is necessary and possibly 3.4 or 3.7 gm. may prove more reliable. This, however, seems to depend to a considerable degree upon the alcohol-stearic-acid solution employed. Some solutions made

from purified alcohol of approximately the same strength require more stearic acid than others to insure a constant saturation, the reason for which we have been unable as yet to determine. Some of the results cited in Tables VI to VIII are probably low, owing to insufficient stearic acid in solution, although the results are all calculated with reference to blank tests conducted under precisely like conditions.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution A (0.8153)[a]	.....	0.3990	0.0525	0.2900	0.2375	0.1077	.....
	.....	.3990	.0500	.2880	.2380	.1073	.....
	.....	.3990	.0500	.2895	.2395	.1063	.....
					[b] .2383		
Solution B (0.8135)[a]	.....	.3960	.0515	.2630	.2115	.1230	.....
	.....	.3960	.0530	.2640	.2110	.1233	.....
	.....	.3960	.0505	.2625	.2120	.1227	.....
	.....	.3960	.0490	.2605	.2115	.1230	.....
	.....	.3960	.0500	.2610	.2110	.1233	.....
					[b] .2114		
Solution C (0.8142)[a]	.....	.4050	.0575	.2770	.2195	.1237	.....
	.....	.4050	.0500	.2710	.2210	.1227	.....
	.....	.4050	.0800	.2990	.2190	.1240	.....
	.....	.4050	.0800	.3005	.2205	.1230	.....
					[b] .2200		
Solution D (0.8142)[a]	.....	.4050	.0820	.3040	.2220	.1220	.....
	.....	.4050	.0805	.3045	.2240	.1207	.....
					[b] .2230		
Solution E (0.8147)[a]	.....	.4470	.1115	.3765	.2650	.1213	.....
	.....	.4470	.1115	.3765	.2650	.1213	.....
					[b] .2650		
Solution F (0.8147)[a]	.....	.4440	.1105	.3840	.2735	.1137	.....
	.....	.4440	.1100	.3830	.2730	.1140	.....
					[b] .2733		
Solution A:							
4	.5440	.3990	.....	.2930	.2383	.....	10.06
4	.5170	.3990	.....	.2900	.2383	.....	10.00
4	.5235	.3990	.....	.2915	.2383	.....	10.16
4	.5000	.3990	.....	.2905	.2383	.....	10.44
							[b] 10.17
Solution B:							
5	.5085	.3960	.....	.2460	.2114	.....	6.80
5	.5190	.3960	.....	.2480	.2114	.....	7.05
							[b] 6.93
6	.5230	.3960	.....	.2555	.2114	.....	8.43
6	.5010	.3960	.....	.2520	.2114	.....	8.10
							[b] 8.27
7	.5135	.3960	.....	.2515	.2114	.....	7.81
7	.5230	.3960	.....	.2500	.2114	.....	7.38
							[b] 7.60

[a] Hydrometer reading at 15.50° C. of the alcohol employed.

[b] Average.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
Solution A:	*Gm.*		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
8	0.5170	0.3990		0.2850	0.2383		9.03
8	.5120	.3990		.2840	.2383		8.93
8	.5225	.3990		.2830	.2383		8.56
							[a] 8.84
Solution C:							
9 [b]	.5090	.4050		.2970	.2200		15.13
9	.5155	.4050		.3000	.2200		15.52
9	.5150	.4050		.2970	.2200		14.95
9	.5130	.4050		.2980	.2200		15.20
							[a] 15.20
Solution B:							
10 [b]	.5130	.3960		.3000	.2114		17.27
10	.5050	.3960		.3020	.2114		17.94
Solution C:							
10 [b]	.4995	.4050		.3070	.2200		17.42
10	.5060	.4050		.3075	.2200		17.29
10	.5255	.4050		.3140	.2200		17.89
							[a] 17.56
Solution B:							
11 [b]	.5065	.3960		.2990	.2114		17.30
11	.5150	.3960		.3000	.2114		17.20
							[a] 17.25
Solution C:							
14	.5165	.4050		.2645	.2200		8.62
14	.5070	.4050		.2635	.2200		8.58
14	.5015	.4050		.2625	.2200		8.47
							[a] 8.56
15	.5045	.4050		.2705	.2200		10.01
15	.5060	.4050		.2715	.2200		10.18
							[a] 10.10
16	.5265	.4050		.2665	.2200		8.83
16	.5205	.4050		.2680	.2200		9.22
							[a] 9.03
17 [c]	.5045	.4050		.2965	.2200		15.16
17	.5300	.4050		.2980	.2200		14.72
							[a] 14.94
18 [c]	.5035	.4050		.2945	.2200		14.80
18	.4990	.4050		.2930	.2200		14.63
18	.5105	.4050		.2940	.2200		14.50
							[a] 14.64
19 [c]	.5205	.4050		.2925	.2200		13.93
19	.5110	.4050		.2905	.2200		13.80
							[a] 13.87
Solution D:							
20	.5180	.4050		.2720	.2230		9.46
20	.5025	.4050		.2715	.2230		9.65
							[a] 9.56
21	.5090	.4050		.2630	.2230		7.86
21	.5205	.4050		.2650	.2230		8.07
							[a] 7.97

[a] Average. [b] The cows were fed beef tallow. [c] The cows were fed palm oil.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
Solution F:	*Gm.*		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
I	0.5070	0.4440		[a]0.3850	0.2733		22.03
I	.5225	.4440		.3915	.2733		22.62
							[b]22.33
II	.5205	.4440		[a].3830	.2733		21.08
II	.5215	.4440		.3850	.2733		21.42
							[b]21.25
Solution E:							
III	.5090	.4470		[c].3525	.2650		17.19
III	.5140	.4470		.3525	.2650		17.02
III	.5060	.4470		.3535	.2650		17.49
							[b]17.23
IV	.5015	.4470		[c].3505	.2650		17.05
IV	.5035	.4470		.3520	.2650		17.28
							[b]17.17

[a] Molecular weight of the several precipitates, 284.54.
[b] Average.
[c] Molecular weight of the several precipitates, 284.59.

TABLE VII.—*Amount of stearic acid in the insoluble acids of beef tallow*

Sample No.	Insoluble acids of beef tallow taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution A		0.3990	0.1500	0.3870	0.2370	0.1080	
Do		.3990	.1555	.3930	.2375	.1077	
					[a].2373		
Solution B		.3960	.1520	.3690	.2170	.1193	
Do		.3960	.1550	.3700	.2150	.1207	
					[a].2160		
Solution A:							
1	0.5280	.3990		.3975	.2373		30.34
1	.5155	.3990		.3960	.2373		30.79
							[a]30.57
Solution B:							
2	.5025	.3960		.3740	.2160		31.44
2	.5150	.3960		.3775	.2160		31.36
							[a]31.40

[a] Average.

TABLE VIII.—*Amount of stearic acid in the insoluble acids of palm oil*

Sample No.	Insoluble acids of palm oil taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution C		0.4050	0.1515	0.3745	0.2230	0.1213	
Do		.4050	.1500	.3750	.2250	.1200	
Do		.4050	.2000	.4255	.2255	.1197	
Do		.4050	.2030	.4295	.2265	.1190	
					[a].2250		
Solution C:							
12	0.3405	.4050	.1500	[b].4040	.2250		8.52
12	.4110	.4050	.1510	.4100	.2250		8.27
12	.5205	.4050	.1540	.4265	.2250		9.13
12	.5000	.4050	.1500	.4205	.2250		9.10
12	.5215	.4050	.1500	.4245	.2250		9.49
12	.5135	.4050	.1565	.4275	.2250		8.96
							[a]8.91

[a] Average. [b] Molecular weight of the several precipitates, 284.38.

The stearic acid obtained from the insoluble acids of butter fat by the method described ranges from 7 to 22 per cent, which is considerably in excess of the amount generally credited to the product. The prevailing opinion was supported undoubtedly by the fact that only a small amount of precipitate is obtainable by the Hehner and Mitchell (3) method, as shown by several investigators.

The amount of stearic acid appears to be affected by the feed the animal receives. Samples 9, 10, and 11, averaging 16.67 per cent, were from cows fed beef tallow; samples 17, 18, and 19, averaging 14.48 per cent, were from those fed palm oil; while samples 4 to 8, 14 to 16, 20 and 21, averaging 8.70 per cent, were from those fed a ration low in fat. It is probable that the individuality of the animal and period of lactation also affect the composition. The entire matter of the effect of food as well as other influences upon the chemical character of butter fat is now being further studied.

The stearic acid (8.91 per cent) recovered from the insoluble acids of palm oil exceeded the amount usually reported.

SUMMARY

The results of the determinations of stearic acid in the insoluble acids of butter fat by the method proposed show a higher percentage of stearic acid than has been generally reported. The facts that the results are concordant and that the molecular weight determinations of the crystallized product secured by the proposed method agree closely with the theoretical molecular weight leave no doubt as to the identity and approximate purity of the stearic acid.

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LIFE HISTORY AND HABITS OF TWO NEW NEMATODES PARASITIC ON INSECTS[1]

[PRELIMINARY PAPER]

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INTRODUCTION

While investigating the life history and methods of control of the elm borer (*Saperda tridentata* Oliv.) and the termite (*Leucotermes lucifugus* Rossi) at the Kansas Agricultural Experiment Station, two new nematodes were found, one parasitic on the former and the other parasitic on the latter. One hundred and twenty-one adult beetles obtained from one tree[2] were placed in breeding cages, but in no instance were eggs deposited, and both sexes eventually weakened and died. Examination after death showed that the intestines were so filled with nematodes that in only one female were eggs even developed in the body. The death rate due to nematode parasitization was apparently 100 per cent. Several colonies of *Leucotermes lucifugus* were placed in salve boxes, together with food. Inasmuch as *Saperda tridentata* had shown so high a nematode parasitization, it was naturally suggested that nematodes might be present in the termites. Accordingly a number of these insects were killed and examined, with the result that nematodes were found infesting the head in varying degrees. Of the colonies taken, 76.92 per cent were parasitized with nematodes. The parasitism of the individuals in single colonies ranged from 0 to 100 per cent.

DIPLOGASTER LABIATA

The nematodes were submitted to Dr. N. A. Cobb, of the Bureau of Plant Industry, United States Department of Agriculture, for identification. He found that the nematode parasitizing *Saperda tridentata* was a new species which he named "*Diplogaster labiata*" (fig. 1; 2, *A–H*), and described as follows:

Diplogaster labiata, n. sp. $\frac{1.2\ \ 17.\ \ 21.\ \ '59''\ \ 91.}{2.1\ \ 4.2\ \ 4.2\ \ 4.4\ \ 2.9}$ 0.66 mm. (The formula was derived from a single specimen.) The thin layers of the transparent, colorless, naked cuticle are traversed by fine transverse striæ, resolvable with high powers into rows of dots, more particularly near the head and on the tail, those on the tail being somewhat irregularly placed. The cuticle is also longitudinally striated, and the dots of the transverse striations are coincident with those of the longitudinal striations. The longi-

[1] Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 17. This paper embodies the results of some of the investigations undertaken by the authors in the prosecution of projects Nos. 13 and 101, Kansas Agricultural Experiment Station.

[2] A tent was placed around an elm tree so that all emerging insects might be secured for breeding purposes.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
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Vol. VI, No. 3
Apr. 17, 1916
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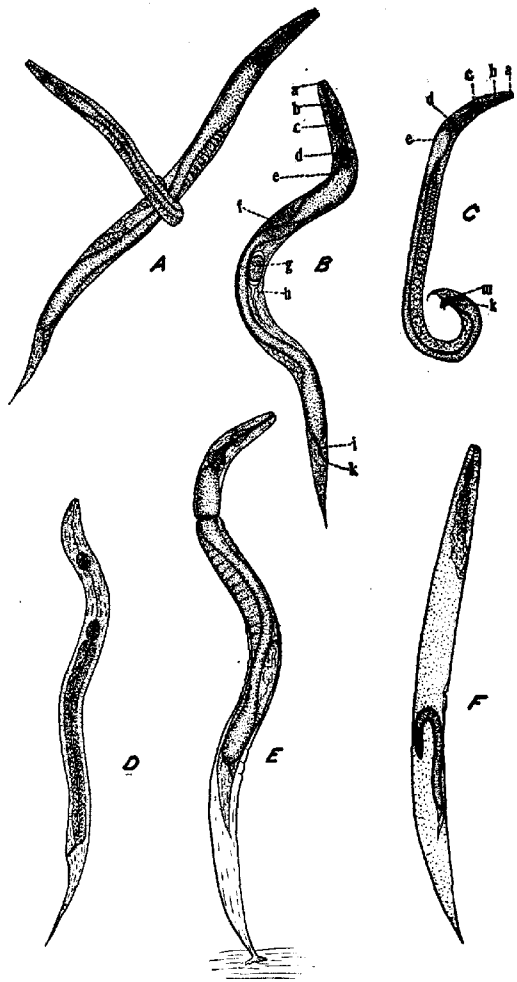

FIG. 1.—*Diplogaster labiata: A*, Mating (× 125); *B*, mature female reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *g*, egg, *h*, genital pore, *i*, rectum, *k*, anus; *C*, mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *D*, at time of hatching (× 400); *E*, female during process of molting (× 125); *F*, dead female with young nematode which hatched within her body (× 125). Drawings by A. L. Ford.

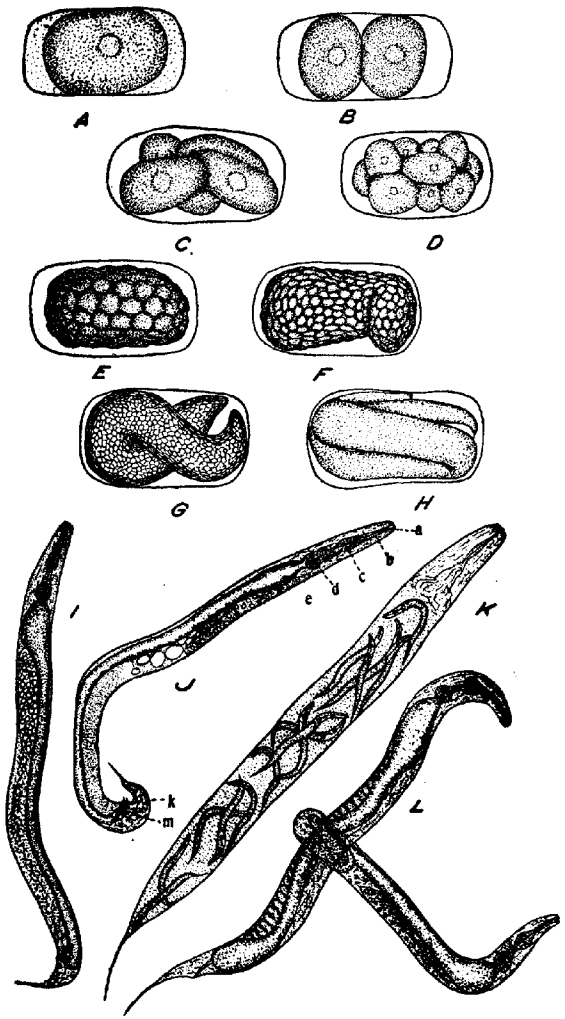

FIG. 2.—*A–H, Diplogaster labiata:* Development of the egg (× 500); *I*, Diplogaster aerivora: mature male reared in moist soil (× 160); *J*, *Diplogaster aerivora:* mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *K*, *Diplogaster aerivora:* dead female with young which hatched within her body (× 125); *L*, *Diplogaster aerivora:* mating (× 125). Drawings by A. L. Ford.

tudinal striæ are not present on the lateral fields, this naked space being one-third to one-half the width of the body. The slightly conoid neck becomes slightly convex-conoid near the head, the lip region of which is set off by a very broad, almost imperceptible constriction. There are six strongly developed and fairly distinct lips, each ending in a conoid tip, from the summit of which issues a very short innervated bristle-like papilla. The lips have a more or less distinct refractive framework and are in all probability quite mobile. Usually in specimens which have been fixed in Flemming's solution the tips of the lips are slightly outward-pointing, leaving a somewhat circular refractive mouth opening about two-fifths as wide as the front of the head. The inner surface of the lips is so strongly refractive that usually the posterior limits of the lips are distinctly visible, more particularly as the wall of the pharynx at this point is encircled by a very delicate refractive line lying considerably in front of the middle of the pharynx. This latter appears to be irregularly cylindroid, but is slightly unsymmetrical at the base. On the whole, it is about two-fifths as wide as the head. It appears to possess at the base a rather well-developed but blunt, slightly inward-projecting process or tooth. In the lateral view, as the posterior part of the pharynx appears to pass around this projection, it acquires the slightly unsymmetrical contour already mentioned. The walls of the esophagus are rather distinctly ceratinized. The esophagus begins at the base of the pharynx as a tube two-thirds as wide as the base of the head and continues to have this diameter, or a slightly greater, until it reaches a point halfway back to the median bulb. Thence onward it diminishes slightly, so that just in front of the median bulb it is only half as wide as the middle of the neck. The median bulb is a well-developed, elongated or ellipsoidal, radially muscular structure, with a somewhat distinct elongated but narrow valve. This bulb is about two-thirds as wide as the middle of the neck. Behind the median bulb the esophageal tube continues with a diameter one-third to two-fifths as great as the corresponding portion of the neck but diminishes very slightly, so that just in front of the ellipsoidal cardiac bulb it is less than one-third as wide as the corresponding portion of the neck. The cardiac bulb contains a rather distinct and rather complicated threefold valvular apparatus and is capable of opening out posteriorly, so that the lumen of the posterior part of the bulb, where it debouches into the intestine, then becomes one-fourth as wide as the corresponding portion of the body. The lining of the esophagus is a distinct feature throughout its length. The intestine, which is thin-walled at first, is separated from the esophagus by a distinct constriction. It becomes at once four-fifths to five-sixths as wide as the body and presents at the beginning a distinct cardiac cavity. There is also a distinct cardia. The cells of the intestine, which are of such size that probably four are required to build a circumference, contain rather large nuclei and are packed with granules of variable size, the largest of which have a diameter as great as the distance between two of the longitudinal striæ, the smallest of which are very much smaller. The lining of the intestine is refractive, so that the lumen is usually quite a distinct feature. From the slightly raised anus the narrow, refractive, ceratinized rectum, which is one and one-half to two times as long as the anal body diameter, extends inward and forward. The tail end begins to taper from some distance in front of the anus but in front of the anus tapers only very slightly. Behind the anus it tapers rather regularly to an acute point. Near the middle of the tail there appears to be a lateral papilla on each side. From the slightly raised, rather broad vulva the vagina leads inward at right angles to the ventral surface nearly halfway across the body, where it joins the two uteri, which extend in opposite directions. The reflexed ovaries reach more than halfway back to the vulva, at any rate in apparently young specimens in which no eggs exist in the uterus. The ova in the ovary are arranged more or less single file for about half its length; toward the blind end they are arranged irregularly. Fertilized females show sperm cells in the uterus of such a

size that about four to five side by side would span the body diameter. Numerous micro-organisms were seen in the intestine.

Male formula. $\frac{1.9}{1.7}\ \frac{16.}{3.1}\ \frac{21.}{3.5}\ \frac{M^{50}}{3.9}\ \frac{94.}{2.5}$ 0.72 mm., single specimen). The tail of the male differs materially in form from that of the female. It begins to taper at the anus, and it tapers rapidly in the anterior two-thirds, more particularly in the middle third, so that at the beginning of the final third it is only about one-tenth as wide as at the anus. Thence onward it tapers rather regularly to the exceedingly fine terminus; there is, however, a pronounced ventral elevation at the beginning of the small part of the tail, though it remains uncertain whether this elevation is innervated. The middle portion of the tail is strongly convex-conoid, the convexity existing largely on the dorsal side. The cuticle of the tail presents a peculiar arrangement of the dots, such that there is an appearance of two sets of oblique fibers crossing each other, these fibers being arranged approximately at 45° to the longitudinal lines. The two equal, rather uniform, somewhat arcuate, blunt spicula are about one and one-fourth to one and one-half times as long as the anal body diameter. Their proximal ends, which are slightly narrower than the main portion, are set off by a rather broad and prominent constriction. At their widest part, through the middle, they are about one-fifth to one-sixth as wide as the corresponding portion of the body. The accessory piece is about half as long as the spicula. It is very inconspicuous near the anus, but lies parallel to the spicula. It widens out to a somewhat clavate or elongated pyriform contour, and has its rounded proximal end toward the dorsal side of the body, and from this blunt end muscular fibers pass obliquely backward to the ventral surface of the tail and join the caudal wall at a distance nearly half way from the anus to the beginning of the narrow portion. Oblique copulatory muscles are to be seen opposite the ejaculatory duct for a distance about one and one-half times as great as the length of the tail. The male papillæ are arranged as follows: One ventrally submedian pair a little in front of the proximal ends of the spicula; one ventrally submedian pair a little in front of the anus, and one ventrally sublateral pair on the same zone; another sublateral pair just opposite the anus; a lateral pair slightly behind the middle of the enlarged portion of the tail; a submedian pair nearly halfway from that last mentioned to the beginning of the small part of the tail; a dorsally sublateral pair a little in front of the beginning of the narrow portion of the tail; three subventral pairs close together opposite that last mentioned; between the members of these three subventral pairs, possibly a single ventral papilla. The most pronounced of these papillæ can hardly be called digitate. The ejaculatory duct is about two-fifths as wide as the body. The vas deferens is nearly two-thirds as wide as the body. The testis tapers so that at the point of inflection, a short distance behind the cardiac bulb, it is about one-fourth as wide as the body. The blind end lies about two body widths behind the flexure.

Habitat: Manhattan, Kans., 1915, on *Saperda tridentata*.

The eggs of *Diplogaster labiata*, elliptical in shape, about twice as long as wide, with bluntly rounded ends, when freshly deposited, were uniformly dark brown or gray, but after segmentation began they became darker. Their average length was 0.0627 mm. and the average diameter 0.031 mm. They were laid singly with apparently no preference as to the place of deposition. Occasionally segmentation began before the eggs were deposited. From the beginning of segmentation the cell divisions could be plainly followed throughout (fig. 2, *A–H*).

A few hours before emerging, the folded young nematodes made slight movements within the egg. Later these movements became

more vigorous until finally they ruptured the shells and emerged, after which the egg walls collapsed. Occasionally a young nematode hatched within the body of a dead female. In cultures the eggs hatched in from 30 to 32 hours from the time of deposition, and the nematodes matured in from 7 to 10 days. The males appeared to mature slightly in advance of the females.

At hatching, the young nematodes were about 0.2 mm. in length (fig. 1, *D*), very slender, and sluggish, and remained for a time in a curled position. Later they straightened out their bodies and became very active. The young worms were almost transparent (in water cultures), there being no solid food in the alimentary canal. As development proceeded, the young became darker in color and more active. At the end of 5 days the sex organs began to appear, and in from 7 to 10 days the nematodes reached maturity.

Specimens which were isolated and kept under observation were noted to molt at least three times, these molts occurring about three days apart. The process of molting (fig. 1, *E*) was as follows: The nematode first fastened its posterior end to any surface upon which it might be resting. The skin then broke at the anterior end and the nematode began to emerge. At first the process was very slow, owing to the fact that the opening of the molt skin was smaller in diameter than the middle part of the body. By moving vigorously from side to side, the nematode slowly worked its way out of the skin. After the widest portion of the body had passed through the opening, no further resistance to emergence was offered, as the posterior end rapidly decreased in diameter. The nematodes were not always able to emerge, as occasionally specimens were found which died before completing the process. Molting lasted from 45 minutes to 6 hours.

The adults and the young were similar in form and food habits, but differed in that the adults possessed sex organs. The mature females were about 0.7 mm. in length and 0.03 mm. in diameter, while the males were about 0.6 mm. in length and 0.02 mm. in diameter.

As soon as maturity was reached, mating began (fig. 1, *A*). The male fastened its caudal end around the middle of the female's body. During this process the male held its body rigid, while the female moved vigorously from side to side. It was not uncommon to find males in the act of mating with their bodies wrapped twice about the females. Toward the end of the process the female increased her activity and soon shook the male free. Many matings were observed, the shortest of which lasted about 2 minutes and the longest 30 minutes.

PROPORTION OF SEXES.—Of 367 specimens examined, 229 were found to be females and 138 were males. In other cultures in which counts were not made the females were noticed to be more abundant than the males.

PERIOD OF OVIPOSITION.—While in the specimens of *Diplogaster labiata* under observation mating usually occurred but once, occasionally a few individuals mated a second time. Oviposition began from two to four hours after mating and lasted over a period of about two days, during which time the average number of eggs deposited was seven.

HABITS.—These nematodes infested the intestines of adults of *Saperda tridentata* in such large numbers that they prevented these insects from performing their natural functions. They lived in the alimentary canal in such large numbers that they ruptured the walls of the canal and, escaping into the body cavity of the insect, caused its death.

The examination of individuals of *Saperda tridentata* which had died in this manner rarely showed eggs that had started to develop. Specimens of *Diplogaster labiata* placed in water cultures were fed on macerated bodies of *Saperda tridentata*. They flourished on this, but since the supply was soon exhausted, substitute foods had to be used. Different substances were tried with varying success, but macerated beetles placed in water seemed to be the most satisfactory. Nematodes in cultures without food usually did not live longer than two days. The presence of food acted as a stimulant to copulation and oviposition, but both varied directly with the abundance and adaptability of the food.

The nematodes seemed to show no preference to either day or night for depositing their eggs or any other of their habits.

LENGTH OF ACTIVE BREEDING STATE.—If the nematode is considered to be mature from the time of mating, it spends an average of about two days as a normal active breeding adult.

DIPLOGASTER AERIVORA

In 1856, Charles Lespés[1] gave a meager description of a nematode which he found parasitizing *Leucotermes lucifugus*. His description is short and so indefinite that it might apply to several species of nematodes, but the habits he discusses closely resemble those of the nematodes found in *L. lucifugus* in Kansas. However, Dr. Cobb identified this nematode as *Diplogaster aerivora* (fig. 2, *I–L;* 3) and described it as follows:

Diplogaster aerivora, n. sp. $\frac{.8\ \ 8.9\ \ 12.}{1.6\ \ 3.9\ \ 4.9}\ \frac{'51^{mm}\ \ 87.}{5.9\ \ \ 2.6}$ 1.5 mm. The transparent, moderately thin layers of the colorless naked cuticle are traversed by fine transverse striæ, resolvable with high powers under favorable conditions. The cuticle is traversed also by 24 longitudinal striæ. These longitudinal striæ are sometimes resolvable into quadrate elements, each consisting of four punctations arranged in a quadrangle whose width is equal to the width of the stria. In the majority of specimens these quadrate elements were not to be seen. The distance between the striæ varies in different parts of the body up to about twice their width. The striations of the cuticle, both transverse and longitudinal, vary within pretty wide limits, the varying

[1] Lespés, Charles. Sur un nématoïde parasite des Termites. *In* Ann. Sci. Nat. Zool., s. 4, t. 5, p. 335–336. 1856.

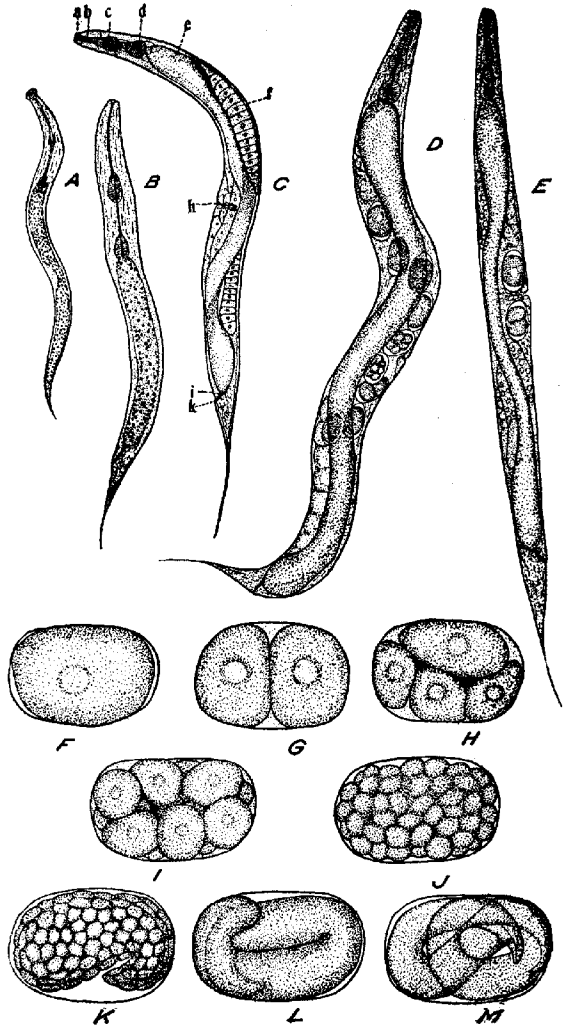

FIG. 3.—*Diplogaster aerivora*: *A*, Form found in termite (× 150); *B*, at time of hatching (× 400); *C*, female reared in water culture, not quite mature (× 100), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *h*, genital pore, *i*, rectum, *k*, anus; *D*, mature female reared in moist soil (× 75); *E*, mature female reared in water culture (× 125); *F–M*, development of the egg (× 500). Drawings by A. L. Ford.

conditions evidently being a function among other things of the age or condition of the cuticle. There are lateral wings, though these consist simply of a pair of slightly modified longitudinal striæ.

The conoid neck becomes convex-conoid toward the truncated head, which is not set off in any way. There are six comparatively well amalgamated lips, each of which bears two innervated papillæ, one on the forward surface and somewhat forward pointing, and one on the outer surface and somewhat outward pointing. The anterior of these two papillæ is extended beyond the surface of the lip in the form of a minute seta or innervated papilla, and corresponds to the cephalic seta of other species of Diplogaster. The contour of the lip is not much disturbed by the presence of the posterior papilla, which is sometimes very difficult to see. Close behind the lateral papillæ or setæ there are minute openings in the cuticle, which in character closely simulate the amphids in some other species of Diplogaster, notably those of *D. fictor*. No doubt these are really the outward expression of minute amphids. Distally the lips have thin extensions which can close together over the pharynx in such a fashion that the front of the head is comparatively flat, though the tips of these lips may be recurved and point forward so as to make an exceedingly minute elevation at the middle of the front of the head. The latter has its front surface on the whole very slightly depressed.

The pharynx is about as deep as the front of the head is wide, and bears near its base on the dorsal side a relatively large, rather acute movable conoid tooth or onchus, which reaches about one-third the distance to the lips when the latter are closed, but which is relatively farther forward when the mouth is open. In addition there is a very much smaller submedian projection that undoubtedly may be denominated a rudimentary onchus. When the lips are closed the pharynx is a little wider at the base than anteriorly. At the base of the lips, opposite the posterior circlet of labial papillæ, the width of the pharynx is a little more than one-third that of the corresponding part of the head. Posteriorly, however, the width appears to be nearly three-fifths that of the corresponding portion of the head, at least when the head is viewed in profile. The walls of the pharynx are thin but refractive and fairly well ceratinized. The surface of the dorsal onchus is more highly ceratinized than that of other portions of the pharynx. Both the onchus and the wall of the pharynx have a yellowish or brownish color like that of the spicula. The end of the esophagus receives the base of the pharynx and is at once fully two-thirds as wide as the corresponding portion of the head. It continues to have the same diameter for some distance, then begins to expand and continues to do so to somewhat behind the middle of the neck, where it rather suddenly diminishes in diameter in such a way that it is proper to speak of a median bulb, although the anterior end of this bulb is not very distinctly set off by constriction from the anterior esophageal tube. This bulb contains an elongated valvular apparatus which is about one-third as wide as the bulb itself. This latter is three-fourths as wide as the corresponding portion of the neck. Notwithstanding the rather massive character of this median bulb, the succeeding portion of the esophagus is only about one-fourth as wide as the corresponding portion of the neck. However, it soon begins to widen and forms a somewhat pyriform cardiac bulb three-fourths as wide as the base of the neck. This bulb does not contain any very evident valvular apparatus, though in it there are faint indications of a modification of the esophageal lining. The intestine joins the posterior surface of the cardiac swelling, and at this point is about one-third as wide as the corresponding portion of the body. There is no very distinct cardia. The intestine widens out rather gradually and attains a width at least half as great as that of the body.

The tail end of the female begins to taper from some distance in front of the anus. This latter is slightly raised, especially its broader posterior lip. Behind the anus the tail diminishes somewhat more rapidly for a short distance and thereafter tapers regularly to the hairfine terminus. From the anus the rectum, which is about as long

as the anal body diameter, extends inward and forward. Nothing definite is known with regard to the lateral fields.

From the well-developed, slightly depressed vulva the vagina leads inward at right angles to the ventral surface halfway across the body, where it joins the two symmetrically placed uteri. The internal female organs are double and reflexed, and the ovaries, which are rather narrow and packed with small ova arranged irregularly, reach back to the vulva or even beyond. The ellipsoidal eggs are about as long as the body is wide and about two-thirds as wide as long. Their shells are smooth and rather thick. Specimens have been seen in which well-developed embryos existed in the eggs contained in the uteri. Other specimens have been found in which two to three dozen embryos had escaped from the eggs and then devoured the whole interior of the mother's body. The excretory pore is located opposite the cardiac swelling.

Male formula. $\frac{.9}{2.2}\ \frac{11.}{5.4}\ \frac{15.}{6.1}\ \frac{M^{88}.}{10.}\ \frac{89.}{4.6}$ 0.8 mm. The tail of the male diminishes suddenly in diameter from the raised anus in such fashion that at a distance from the anus not very much greater than the anal body diameter it has a diameter only about one-fourth to one-fifth as great as at the anus. At this point, which is immediately behind the posterior group of male papillæ, the tail begins to taper rather gradually and somewhat uniformly, and continues so to do to the hairfine terminus, though there is at first a very slight increase in the diameter, so that the tail has the appearance of being very slightly constricted just behind the posterior caudal group of male papillæ. There is no spinneret, and there are no caudal glands. The two equal, rather slender, tapering, arcuate, brownish, acute spicula are about one and one-half times as long as the anal body diameter. At their widest part, a little distance behind the cephala, the spicula have a width about one-tenth as great as that of the corresponding portion of the body. From this widest part they taper gently toward the cephalated proximal ends. In the other direction the spicula taper regularly to their acute terminals. The accessory pieces surround the spicula at their distal extremities. The portion of the spiculum surrounded by the accessory piece constitutes about one-sixth of the length of the former. Extending backward from this encircling part of the accessory piece is a median arcuate portion arranged nearly parallel to the spicula and having its proximal end somewhat cephalated. The entire length of the accessory piece, including this median dorsal portion, is about one-third that of the spicula. Like the spicula the accessory pieces are brownish in color.

The hemispherical-conoid innervated supplementary male organs are located as follows: In front of the anus three pairs, two of which are ventrally submedian and one sublateral; the sublateral pair is nearly opposite the middle of the spicula, and is on nearly the same zone as the posterior of the two ventrally submedian pairs; the anterior submedian pair is a little in front of the proximal ends of the spicula. Behind the anus the papillæ are arranged as follows: One pair subventral or ventrally submedian immediately behind the anus, two pairs sublateral, and three closely approximated pairs of small size, subventral. This latter group of three pairs is slightly farther behind the anus than the foremost preanal pair is in front of it. The three pairs do not appear to be uniform in structure, the two anterior appearing to be mere innervations, while the posterior one is a distinctly raised innervated papilla like the preanal ones. The posterior of the two pairs of sublateral postanal papillæ is a trifle in front of the group of three just mentioned, while the anterior is about halfway between the group of three and the anus. The anterior border of the anus constitutes a sort of rudimentary flap with an innervation. The testis is single and rather broad and tubular. It extends forward and is reflexed a short distance behind the base of the neck. The reflexed narrower part of the testis is about twice as long as the corresponding body diameter.

Habitat: Manhattan, Kans. Found feeding on grasshopper eggs after the eggs had been deposited in the ground.

The eggs of *Diplogaster aerivora*, which are elliptical in shape, averaged about 0.062 mm. in length and 0.0335 mm. in diameter. When freshly deposited, they were dark brown in color, but became transparent as the embryo developed. Segmentation often began before the eggs were deposited and the succeeding cell divisions could (fig. 3, *F–M*) be readily followed throughout. The eggs were numerous and could be found lying close together in groups of from about 6 to 30. The eggs hatched in about 18 hours from the time segmentation was first noticed. Toward the end of the egg stage the living worm (fig. 3, *M*) could be plainly seen moving about within the egg wall. These movements became more active until the worm finally ruptured the wall and escaped.

At the time of hatching, the young nematodes (fig. 3, *B*) of this species averaged 0.2145 mm. in length. At this stage the sex organs could not be distinguished, because of their poor development. In water cultures the worms grew very rapidly and reached maturity in three to four days. The females matured slightly in advance of the males (fig. 2, *J*). *D. aerivora* never exceeded 0.5 mm. in length nor completed its life cycle while within the termite (fig. 3, *A*). The nematodes remained in the termite in this form for an indefinite length of time, but upon emerging into moist soil they matured in about two days.

Although molting occurred in this species as in *D. labiata*, it was much more difficult to observe; and, while it was not observed more than once in any individual, it is probable that more molts did occur. Molting required less time in *D. aerivora* than in *D. labiata*, and the posterior end of the nematode remained free throughout the process.

In the older water cultures the adults became so numerous that they appeared as a living mass to the naked eye. The females, which were much larger than the males, averaged 0.99 mm. in length and 0.067 mm. in diameter, while the males averaged 0.75 mm. in length and 0.046 mm. in diameter. When free in moist soil, the worms became even larger; the females (fig. 3, *D*) averaged 1.632 mm. in length and 0.1192 mm. in diameter, and the males (fig. 3, *E*) averaged 1.1425 mm. in length and 0.0724 mm. in diameter.

When reared in water cultures, the females appeared darker than the males, but when found in the soil both sexes appeared pearly white. The alimentary canal of the female, like that of *D. labiata*, was spiral, while that of the male was straight. The posterior end of the female's body tapered into a long, threadlike process, but in the male this process was shorter and its body ended in an abrupt hook.

PROCESS OF MATING.—The process of mating in *D. aerivora* (fig. 2, *L*) was much the same as in *D. labiata*. The male clasped the female slightly back of the middle of the body, so that its anal opening was in direct apposition to the genital pore of the female. In mating, the posterior end of the male usually completely circled the body of the female, although exceptions occurred. Although many instances of mating

were observed, none lasted over 4½ minutes. As the mating neared completion, the female became more active and broke free.

RELATION AND ECONOMY OF THE SEXES.—Both males and females mated repeatedly with different individuals. A single female was observed to mate with 7 different males, and during this time laid a total of 317 fertile and 14 infertile eggs. The length of time from the first to the last mating was 13 days. The greatest number of fertile eggs produced from a single mating by any individual under observation was 125, but the average number was 52.63. A single male was successfully mated with 10 different females, the latter depositing 624 fertile eggs. The total time which elapsed during these 10 matings was 19 days.

TIME AND METHOD OF OVIPOSITION.—A single instance was observed of a female depositing a fertile egg 30 minutes after mating, although from one to two hours are usually required. The eggs developed in the ovaries in large numbers and were rapidly discharged through the genital pore. With age the females became very sluggish and did not appear to be able to discharge their eggs; consequently these eggs hatched within the body of their parent, where they fed on her internal organs. Usually they were unable to escape, although instances were observed where they escaped through the genital pore of the mother (fig. 2, *K*).

PROPORTION OF SEXES.—Three hundred specimens were examined, and of these 138 were males and 162 were females. In all cultures the females seemed to be more abundant.

HABITS.—These nematodes were found parasitic in the heads of *Leucotermes lucifugus*, where under natural conditions the number varied from 0 to about 75. Where heavy infestation occurred, the termites became sluggish and often died. These worms were usually more numerous in the immediate region of the mouth parts of *Leucotermes lucifugus*, although it was not uncommon to find them in the upper part of the cavity of the head. A great many termites were dissected, and in no case were nematodes found in the abdomen. In infested colonies nematodes were often seen in the surrounding soil. These usually were found in masses, feeding upon the bodies of dead termites or other available decaying matter. Specimens of *D. aerivora* placed in water cultures were found to flourish in the same food that was used for *D. labiata*. It was necessary to feed these nematodes each day, for without food they died in a very short time. As in *D. labiata*, the presence of food appeared to stimulate copulation and consequently caused an increase in oviposition.

So far as could be determined, these nematodes showed no preference to either day or night in mating, oviposition, or other habits.

LENGTH OF ACTIVE BREEDING STAGE.—The active breeding life of the female extended over a period of about 13 days, while that of the male was about 19 days. The complete life cycle of *D. aerivora* required from four to five days. As the individuals of this species which were

examined had no hibernation stage, their life cycle was continually repeated under favorable conditions. Insufficient moisture and lack of suitable food seriously interfered with the development of these nematodes.

A series of experiments was carried on to ascertain whether it is possible to introduce these parasites into *Leucotermes lucifugus*. Good cultures of nematodes were obtained in moist soil, into which specimens of *L. lucifugus* were placed. After two days a number of these termites were dissected, and it was found that there was an average of 22.9 nematodes in each head. In three days this average rose to 32.9 and in four days it was 46.6. In each instance the check count remained the same, being about 3 nematodes per head. After remaining in a similar culture for 12 days, all the termites died and the bodies were found to be literally alive with nematodes.

SUMMARY

(1) The eggs of *Diplogaster labiata* hatched in from 30 to 32 hours, while those of *D. aerivora* hatched in about 18 hours.

(2) The eggs of *D. labiata* were deposited singly, while those of *D. aerivora* were deposited in groups.

(3) More cases of eggs hatching in the body were found in *D. aerivora* than in *D. labiata*.

(4) The eggs of both species developed similarly.

(5) Both species, when reared in water cultures, used the same food, but in nature they had different hosts.

(6) Both species molted, but the process differed in that *D. labiata* fastened its posterior end, while *D. aerivora* did not.

(7) The adults of *D. aerivora* were larger than those of *D. labiata* and required much less time to mature.

(8) In water cultures, the females of both species were more numerous than the males.

(9) Although mating was similar in both species, *D. labiata* required more time for the process.

(10) Individuals of *D. labiata* usually mated but once, while those of *D. aerivora* mated repeatedly.

(11) Neither species in their habits showed any preference to day or night.

(12) The females of *D. aerivora* had a period of oviposition of about 13 days, while in *D. labiata* this period lasted only about 2 days.

(13) In both species adaptable and plentiful food acted as a stimulant to reproduction.

(14) Both species attacked insects, but in different regions of the body, as *D. aerivora* was found in the head while *D. labiata* was found in the intestines.

(15) The life cycle of *D. labiata* required more than twice as much time as did that of *D. aerivora*.

(16) *D. aerivora* was successfully introduced into the termites.

INSECT INJURY TO COTTON SEEDLINGS[1]

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INTRODUCTION

The present work deals with leaf mutilation of cotton seedlings (*Gossypium* spp.) caused by insects. The observations were made in the vicinity of Tallulah, La., during the spring of 1915. Such injury to cotton seedlings is probably found throughout the entire area of cotton cultivation in the United States. The senior author has noted it in many parts of Texas, both the drier and more humid portions, in Louisiana, and in Arizona on irrigated cotton. Since these localities approximate the extremes of rainfall, temperature, and sunshine under which cotton is cultivated, it is reasonable to expect the injury at almost any place.

CHARACTER OF INJURY

The injury varies much in appearance and intensity, but all of the examples which have come to the attention of the authors have certain more or less constant characteristics. This is frequently noticed as soon as the seedlings appear above the ground, although it may not appear until later. The time of the cessation is also variable, but it does not seem to continue after the plants reach a height of 10 to 12 inches and usually stops much earlier. In the vicinity of Tallulah this injury is seen from the first sprouting of the plants until the latter part of May.

The first appearance is characterized by irregular holes appearing in the cotyledons. These vary from small holes through the leaf or small marginal incisions to almost complete loss of the leaf. Following this the later leaves are attacked in the same manner, with all possible variations in the type and degree of the injury. In some cases the terminal bud may be lost.

LABORATORY STUDIES

Efforts were made to secure growing plants at the earliest possible date. For this purpose cotton seed was planted in boxes and pots in the laboratory during the very early spring, but lighting facilities were so poor at this season that the plants failed to thrive. The first healthy seedlings which were secured sprouted in the laboratory hotbed March 16 from seed planted in the middle of February. Seed planted in another part of this hotbed on March 5 sprouted well a little later. This hotbed

[1] The investigations upon which this paper is based were conducted under the direction of Mr. W. D. Hunter, in Charge of Southern Field Crop Insect Investigations, Bureau of Entomology.

Journal of Agricultural Research, Dept. of Agriculture, Washington, D. C.
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Vol. VI, No. 3
Apr. 17, 1916
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was covered with glass during the night and was only opened during the warmer part of the day. The plants appeared perfectly healthy at all times and grew well.

Other plantings were made in the laboratory yard at intervals during March for studies under outside conditions. Later, seeds were germinated between layers of moist absorbent cotton and placed in pots containing soil sterilized by baking. These pots were then placed in large screen cages and the plants were allowed to grow under this protection.

The first injury was noted in the hotbed on March 31. These seedlings had sprouted March 16 and at this time were about 3 inches tall. They had been protected from cold by the glass covers, and the soil had been well manured. On this first morning a number of plants were found to have been injured.

Following this the progress of the injury was noted carefully. All plants were examined daily and those showing injury were tagged. In this manner a record of the number of plants injured each day was secured. On the morning of April 14 nine new seedlings were injured; on April 15 five, on April 16 six, on April 17 two, and on April 18 three.

In order to determine the period in which the injury was incurred, both morning and evening counts were started. These showed the number of seedlings injured during the night and during the day. These observations were started April 22 and continued until May 4. The results are presented in Table I.

TABLE I.—*Comparison of day and night injury to cotton seedlings*

Date of examination.	Number of seedlings injured during day.	Number of seedlings injured during night.	Date of examination.	Number of seedlings injured during day.	Number of seedlings injured during night.
Apr. 22		5	May 1	0	1
23	7	6	2	0	3
26	4	10	3	0	4
27	2	2	4	0	3
28	4	5			
29	4	4	Total	26	49
30	5	6			

From this table it is seen that 66 per cent of the injury appeared during the night and 34 per cent during the day.

On April 14 this same type of injury appeared upon seedlings which had just sprouted in the laboratory garden, and from that time it appeared about as abundantly here as in the hotbed.

The rapidity with which the injury was produced was quite striking, and special studies were made upon this point. A number of apparently healthy and entire seedlings were examined morning and evening, and in that way the amount of injury produced in a single night was

determined. This was done in both the hotbed and the garden, and the results were the same in both cases. Leaves which were entire and uninjured at nightfall would show large holes often occupying one-half of their area on the following morning. Later observations have shown practically entire leaves disappearing in the same manner during the night.

During the first few days when the injury was appearing in the hotbed a number of examinations were made during the daytime in the attempt to find some insect producing the injury, but not a single individual which could be suspected of being the cause was noted. However, on April 6, 50 square inches of the hotbed soil were examined to a depth of 3 inches, and 12 cutworms were found. If this was a fair sample of the hotbed, the soil there certainly contained hundreds of the worms. Eleven of these larvæ were very small, while one was about an inch in length.

The presence of these larvæ in the hotbed and the fact that they were known to feed upon plant leaves made it seem quite possible that they were responsible for more or less of the injury. Consequently several examinations were made at night, and a number of cutworms were found feeding on the leaves of the plants. At this time the same injury was noted on clover and weed leaves in the hotbed.

Several half-grown cutworm larvæ collected on cotton in the garden and hotbed were placed on the surface of the soil in a pot containing a number of seedlings. This pot was placed in a screen cage and the larvæ attacked the seedlings at once. Plate XII, and Plate XIII, figure 1, show several seedlings injured by these larvæ.

STUDIES OF CLIMATIC FACTORS

A number of tests were conducted to determine whether any of the injury could be due to the exposure to low temperatures during the night or to the hot sunlight in the morning before the plants had time to become warm. In the first test a wooden frame was erected over a cotton planting in the laboratory garden just prior to the sprouting of the plants. This frame was 2½ feet in height and was covered with 8-ounce duck. This cloth was placed over the frame at sundown each day and allowed to remain until about 10 o'clock the following morning. In this manner the radiation was reduced under this cover during the night and the plants were protected from sudden exposure to the sunlight in the early morning. A minimum thermometer was suspended under the cover in the center of the bed about 15 inches from the ground and another was suspended at the same height in the open garden a few feet away. Records continued for a few nights showed only a slightly higher temperature under the shelter, so the frame was lowered to within 1½ feet of the ground and the thermometers were lowered to 6 inches. Following this the minimum temperatures under the cover usually ranged a few degrees higher than in the open.

This frame was first erected April 26 and on April 30 the first seedlings appeared above the ground. Of the 18 which sprouted this first day, 5 showed injury. On May 1, 9 of the 45 seedlings showing above the ground were injured, while on May 3, 10 out of 50 were injured. On May 4, 16 out of 70 and on May 5, 22 out of 70 were injured. These observations were continued until May 8 and new seedlings were injured practically every day.

On May 8 a second test of the same sort was started. In this case, however, the cotton row was covered just before sprouting with heavy pasteboard boxes, 1 foot square and 8 feet long. These boxes were covered with several layers of 8-ounce duck and were only removed from over the plants during the hotter part of the day. Minimum thermometers were arranged under the boxes and in the open in the same manner as that just described in the preceding test. In this case considerable differences in the nightly minimum temperatures were noted. It was usually from 3 to 6 degrees warmer under the box than in the open. On May 12 the first seedlings appeared, and of the 39 in sight, 3 showed injury to the leaves. This test was continued six days longer and the injury continued to appear.

For comparison with the seedlings growing in the garden and hotbed, a number of seeds were planted at intervals in pots and crocks containing soil sterilized by baking. Part of these were allowed to remain exposed in the open, while others were placed in screen cages. In the hundred or more seedlings grown in this manner not a single sign of injury was found, whereas the injury was appearing abundantly on plants growing in the garden and hotbed at this same time. From this it seemed quite evident that the cause of the injury was located in the soil which had not been baked.

FIELD OBSERVATIONS

As the injury was appearing in the various fields at this same time, efforts were made to learn its extent and to discover any insects which might cause the lesions. In these studies all insects which were known to be leaf feeders were noted and an attempt was made to secure positive samples of their injury to cotton. On April 19 four small lepidopterous larvæ were found feeding upon the leaves of cotton seedlings at a plantation near Tallulah. The injury which they were producing was apparently identical with that already noted. These larvæ belong to the family Liparidae and are commonly known as "tussock moths" (*Hemerocampa leucostigma* Smith and Abbot). On this same date three larvæ of the same species were found feeding on the seedlings in the hotbed and one was found in the laboratory garden. Following this the field examinations showed a considerable number of these larvæ to be present around Tallulah, and associated with them were found several species of cutworms and "measuring worms." All produced nearly the same type of injury to the seedlings.

In order to determine definitely the amount of injury present in the various cotton fields around Tallulah and also the prevalence of the worms, a considerable number of examinations were made during the latter part of April. In these observations only the worms found on the cotton seedlings were noted. In order to make the figures more accurately represent the condition of the field, the plants were examined in groups of 100 each in all parts of the field. The results are summarized in Table II.

TABLE II.—*Records of examinations for insect injury to cotton seedlings in fields around Tallulah, La.*

Date.	Number of seedlings examined.	Number of seedlings injured.	Per cent of seedlings injured.	Number of lepidopterous larvæ found.	Type of soil.	Remarks.
Apr. 20	1,000	266	26.6	25	Sandy	All tussock larvæ; very small.
21	200	30	15.0		do	Seedlings just above the ground.
22 and 23	2,300	534	32.2	25	do	Eleven cutworms and 14 tussock larvæ.
22	1,000	84	8.4	1	Buckshot	Cutworm.
22	800	188	23.5	1	Sandy	Do.
22	800	54	6.7		Buckshot	
24	2,300	207	13.4	9	Sandy	Six cutworms and 3 tussock larvæ.
27	1,200	380	31.7	4	do	Two geometrid larvæ and 2 tussock larvæ.
27	1,000	227	22.7	1	do	Tussock larva.
29	400	43	10.7		do	
Total	11,000	2,013		66		Forty-five tussock larvæ, 19 cutworms, and 2 geometrids.
Weighted average			18.3			

From this it is seen that the percentage of plants injured at the various plantations visited ranged from 6.7 to 32, with an average of 18.3 per cent for the 11,000 seedlings examined. In the course of these observations 66 lepidopterous larvæ in all were found. By far the greater part of these were the "tussock" larvæ and the remainder were either cutworms or "measuring worms."

The possibility of the soils having some influence upon the extent of damage was considered, but the writers were unable to secure sufficient information to allow definite conclusions. Soils in the vicinity of Tallulah may be roughly classed as either "sandy" or "buckshot." The former is the light, sandy land found on the bayou fronts, while the "buckshot" is the dark, heavy, stiff "back land." Under boll-weevil conditions "buckshot" land is not adapted to cotton culture; hence, only two fields of this type of soil were located for study. The percentage of injured seedlings in these two fields was 6.7 and 8.4. These were the lowest records made and are considerably below the average of sandy fields near by. Whether or not this lesser degree of injury was due to the soil is open to doubt. Owing to the "coldness" of "buckshot" land in the spring, the cottonseed germinates slowly and consequently the plants were considerably smaller

than those on sandy land. This may have caused the difference in the percentage of injury. However, only one suspected larva (a cutworm) was found in the two fields.

The different lepidopterous larvæ noted were all observed to be feeding upon the leaves. The tussock larvæ were much the more abundant and evidently produced a great deal of the injury. During the earlier examinations nearly all of these tussock larvæ were quite small. The injury produced varied somewhat with the size of the larva. The very small individuals fed only upon the epithelium of the lower side of the leaf and the injury was not visible from above. With a slight increase in size the larvæ started to feed through the leaf and at this stage produced the peculiar type of injury shown in Plate XIII, figure 2. Later the older larvæ (one-half to full grown) ate large holes in the leaves, and the injury could no longer be distinguished from that of the other species concerned. Plate XIII, figure 3, shows the injury produced by one nearly full-grown tussock larva when confined in a large screen cage with cotton seedlings growing in a pot.

About May 1 nearly all cotton fields under observation suddenly began to show greatly increased injury until within a few days many fields had practically every plant more or less mutilated. This proved to be due to an invasion of grasshopper nymphs. These speedily became very abundant and swarmed over the young cotton, feeding principally upon the leaves. This is shown in Plates XIV and XV. These cotton leaves were collected in the field when the young grasshoppers were feeding upon them.

A little later in May the 12-spotted cucumber beetle, or adult of the southern corn rootworm (*Diabrotica 12-punctata* Olivier), became abundant locally and added to the injury. The work of these beetles closely resembled that of the worms and grasshoppers, though the holes made were usually not very large. At this same time woolly-bear larvæ began to appear in the fields and produced the same injury.

Following this great increase in injury to the plants caused by the grasshoppers, counts were made to determine the percentage of injured seedlings in four average fields near Tallulah. The information secured from these examinations is shown in Table III.

TABLE III.—*Abundance of injured cotton seedlings after the grasshopper invasion*

Date.	Number of seedlings examined.	Number of seedlings injured.	Percentage injured.
May 14	800	792	99.0
15	3,500	3,446	98.5
17	2,000	1,920	96.0
17	1,000	1,000	100.0
Total	7,300	7,158	
Weighted average			98.0

Here it is seen that 98 per cent of the 7,300 seedlings examined had been injured by some of the various agencies operating prior to that time. High as they are, these figures are representative of average conditions in the fields near Tallulah.

ACTIVE PERIOD OF LARVÆ

On April 14 continuous examinations of cotton seedlings were made from 8 a. m. until noon and from 1 to 5 p. m. on two plantations near Tallulah. The day's records of worm collections were divided into hourly periods and in this manner the active time of the various larvæ was noted. The results of these studies are shown in Table IV. From this it is seen that the tussock larvæ were much the more abundant throughout the day and there seemed to be no time at which they were especially abundant on the plants. The same seems to be true of the other larvæ.

TABLE IV.—*Records of field examinations for larvæ by hourly periods on two plantations near Tallulah, La.*

Period.	Number and kinds of larvæ found.	
	First plantation.	Second plantation.
8 a. m. to 9 a. m.	2 tussock larvæ, 1 cutworm.	1 tussock larva.
9 a. m. to 10 a. m.	7 tussock larvæ.	4 cutworms, 2 yellow "woolly-bear" larvæ.
10 a. m. to 11 a. m.	9 tussock larvæ.	9 tussock larvæ, 2 cutworms, 3 yellow "woolly-bear" larvæ.
11 a. m. to 12 noon.	3 tussock larvæ, 2 small cutworms.	No examinations.
1 p. m. to 2 p. m.	7 tussock larvæ, 1 small cutworm, 1 yellow "woolly-bear" larva.	No worms.
2 p. m. to 3 p. m.	6 tussock larvæ.	2 unknown larvæ.
3 p. m. to 4 p. m.	6 tussock larvæ, 1 small cutworm.	3 unknown larvæ.
4 p. m. to 5 p. m.	7 tussock larvæ, 2 yellow "woolly-bear" larvæ.	1 tussock larva, 1 geometrid, 6 unknown larvæ.
Summary.	57 tussock larvæ, 5 cutworms, and 3 yellow "woolly-bear" larvæ.	14 tussock larvæ, 8 cutworms, 5 yellow "woolly bears," 1 geometrid, and 11 unknown larvæ.
Total, both plantations.	71 tussock larvæ, 13 cutworms, 8 yellow "woolly-bear" larvæ, 1 geometrid, and 11 unknown larvæ.	

INJURY TO TERMINAL BUDS

The greater part of the feeding of the insects just mentioned is confined to the leaves. However, a considerable number of plants were found with the terminal buds either partially or completely destroyed. Plate XVI, figure 1, shows the usual location of this injury. This seedling was found in the field with a lepidopterous larva embedded at the base of the bud (*a*). The small cavity where the larva was feeding is shown in the photograph. From this the injury progresses until often all the buds and small leaves above point *a* are eaten out.

ULTIMATE EFFECT OF INJURY UPON THE PLANTS

The preceding pages have shown the different insects contributing to the mutilation of cotton seedlings, but it is the ultimate effect upon the cotton production of the plants which determines the economic importance of the injury. This is a point upon which it is difficult to secure accurate data, but a certain amount of information has been gathered by the writers.

A number of plants are evidently killed outright by the feeding of the insects; but this number appears to be so small, even in fields very heavily infested, that it is of no practical importance.

The leaf feeding is also of very doubtful importance. In severe cases it retards the growth of the plants somewhat and occasionally dwarfs them permanently, but usually they recover very rapidly, and there is no visible effect other than the slight retardation.

Apparently it is the injury to the terminal buds which produces the most important economic effect. When this bud is injured or destroyed, the development of the plant is greatly changed. Instead of having a single main stem extending to the top of the plant, two or more large branches develop just below the injured bud and serve as stalks to produce the fruiting branches. Usually several very abnormal clusters of leaves form around the stalk near the injury. In Plate XVI, figure 2, the result of similar injury is shown in comparison with a normal plant. These two plants were collected in the garden at the laboratory and were stripped of their leaves before being photographed. Plant *B* shows a normally developed stalk and its branches, while plant *A* shows the deformity caused by the destruction of the terminal bud.

About the middle of June a number of examinations were made in the fields near Tallulah in order to determine the abundance of these deformed plants. The results of these examinations are given in Table V.

TABLE V.—*Records of field examinations for deformed cotton plants at Tallulah, La.*

Date.	Number of plants examined.	Number of plants deformed.	Percentage deformed.	Location.
June 8	4,000	314	7.8	Plantation.
9	100	3	3.0	Hotbed.[1]
9	100	7	7.0	Laboratory garden.[1]
10	1,000	87	8.7	Plantation.
11	100	4	4.0	Do.
11	500	63	12.6	Do.
16	400	33	8.2	Do.
17	600	42	7.0	Do.
Total	6,800	553	.....	
Weighted average			8.1	

[1] Just prior to this examination the plants in the garden and hotbed had been hand thinned; and as the poorest plants were removed, the percentage of deformed plants was evidently greatly lowered.

From this it is seen that the percentage of deformed plants ranged from 3 to 10.6, with an average of 8.1. As these same fields furnished the records given in Tables II and III, and were shown in the latter to have practically every plant more or less mutilated, it seems evident that only a comparatively small amount of the injury produces final deformity. However, an injury which deforms only 8 per cent of the plants in a field still is of considerable importance.

When this deformity was first observed it was at once noted that the injured plants were not forming as many squares as normal plants of the same age and height. Further studies showed this effect to be so pronounced that counts were made in the fields to determine the relative squaring of deformed and normal plants. In these observations, every time a deformed plant was found its squares were counted, and likewise those on the nearest eight normal plants of the same size. The average of these normal plants was compared with the number upon the deformed one. In 40 cases out of the 229 recorded the squares on the injured plants exceeded the average of the nearby normal plants, but in all others the average of the normal ones was considerably higher than the number on the injured plants. A summary of these observations is given in Table VI.

TABLE VI.—*Effect of deformity upon fruiting of cotton plants*

Date.	Deformed plants.				Normal plants.			
	Number observed.	Total squares.	Average squares per plant.	Maximum squares per plant.	Number observed.	Total squares.	Average squares per plant.	Maximum squares per plant.
June 10	87	248	2.8	10	700	3,804	5.4	16
11	4	23	5.0	9	32	267	8.3	13
11	63	52	0.8	6	502	1,105	2.2	10
16	33	405	12.3	26	264	3,931	14.9	34
17	42	559	13.1	34	336	6,122	18.2	53
Total	229	1,287	.....	.....	1,834	15,229	.....	.....
Weighted averages	.....	.....	5.6	.....	.....	.....	8.2	.....

The 229 deformed plants averaged 5.6 squares per plant, while the 1,834 normal ones averaged 8.2 squares. This gives a difference of 2.6 squares per plant in favor of the normal plants at the time of these observations.

From these figures it is evident that the necessity for the additional vegetative development before squaring retards the fruiting of the plants considerably. This is a point of great importance in cotton culture under boll-weevil conditions. The primary requisite for a successful crop in the presence of the boll weevil is early, rapid, and prolific fruiting. This allows the safe "setting" of a crop before the weevils multiply

sufficiently to infest all the squares. Hence, any agency which retards the formation of the squares in the early spring does a very serious injury to the crop. While the deformed plants may overtake the normal plants later in the quantity of fruit, this fruit will be produced too late to insure safe maturing.

Another effect of the deformity which may be of considerable importance is the ease with which the plants are split when the two or more branches fork at the same point. This gives a very weak stalk, and a comparatively slight jar will split it. In fact, the weight of a crop of bolls will break many of the plants.

SUMMARY AND CONCLUSIONS

From the various observations discussed in this paper it seems that mutilation of cotton seedlings may be produced by any of several insect pests. These consist of a number of species of lepidopterous larvæ (cutworms, measuring worms, "woolly-bear" larvæ, tussock-moth larvæ, etc.), grasshoppers, and leaf beetles. In all fields several species of these pests were present, and in many fields all of them were found. During the spring of 1915 at Tallulah, La., the tussock larvæ were responsible for most of the damage early in the season and then were supplanted by the grasshopper nymphs. However, the relative importance of the various species undoubtedly varies with the locality and season.

Tests made with plants protected from low temperatures during the night and from bright sunshine in the early morning demonstrated that the injury would appear about as abundantly on these plants as on the unsheltered plants in the garden and field. Seedlings in large number, raised through this period in pots and crocks containing baked soil, failed to show the slightest trace of injury, although they were fully exposed to the weather.

Injury to cotton by cutworms has been known for many years, but usually has been considered to consist only of the cutting of the plant stem near the ground. In 1897 Howard [1] published a brief review of the information then available concerning these larvæ, but did not mention them as leaf feeders. In 1905 Sanderson [2] mentioned the injury due to these worms and also discussed the work of *Prodenia ornithogalli*. This species he recorded as being diurnal in habits and feeding upon the leaves, but he considered the damage to the squares and bolls as its most important injury. Sanderson also mentioned the "woolly-bears" as occasionally damaging cotton by feeding upon the leaves.

In actual effect upon the plants it seems that the injury of the various species may result in death of the plant, dwarfing of growth, or deformity

[1] Howard, L. O. Insects affecting the cotton plant. U. S. Dept. Agr. Farmers' Bul. 47, 32 p., 18 fig. 1897.

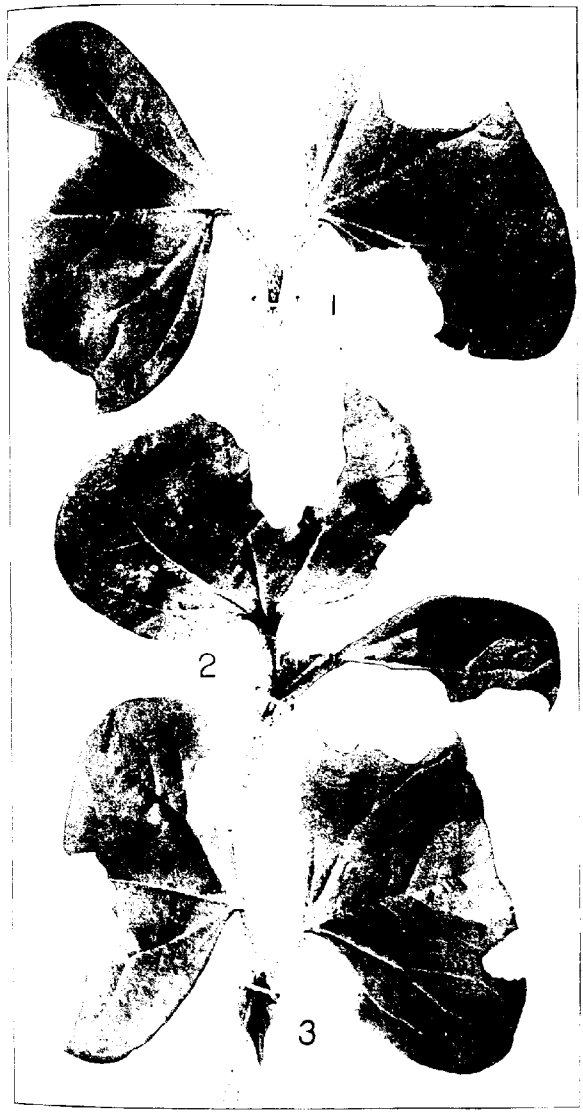
[2] Sanderson, E. D. Miscellaneous cotton insects in Texas. U. S. Dept. Agr. Farmers' Bul. 223, 24 p., 29 fig. 1905.

of the stem, producing retardation of the fruiting. Of these the deforming of the stalk is evidently much the more important. Field examinations have shown that an average of 8 per cent of the plants in the fields under observation were deformed and that these abnormal plants averaged 2.6 squares per plant less than the normal ones about the middle of June. As the cotton in these fields averages about 4 feet between the rows and is spaced about 18 inches in the drill, this would mean a loss of over 1,500 squares per acre at the critical period in cotton production in the presence of boll weevils.

The "woolly-bear" larvæ mentioned in this paper were reared and proved to be *Estigmene acraea* Drury. Two of the cutworms have been identified by Mr. S. E. Crumb, of the Bureau of Entomology, as *Prodenia ornithogalli* Guenée and *Peridroma margaritosa* Haworth, var. *saucia* Hübner.

PLATE XII

Fig. 1.—Cutworm injury to cotton seedlings; produced in breeding cages.
Fig. 2, 3.—Cutworm injury to cotton seedling.


1
2
3


1
2
3

PLATE XIII

Fig. 1.—Cutworm injury to cotton seedling.

Fig. 2.—Tussock larva feeding upon cotton leaf. The ragged injury shown here is usually produced by the smaller larvæ.

Fig. 3.—Injury produced by a nearly full-grown tussock larva when confined in a screen cage containing potted cotton plants.

PLATE XIV

Cotton leaves showing grasshopper injury.


1
2


1
2

PLATE XV

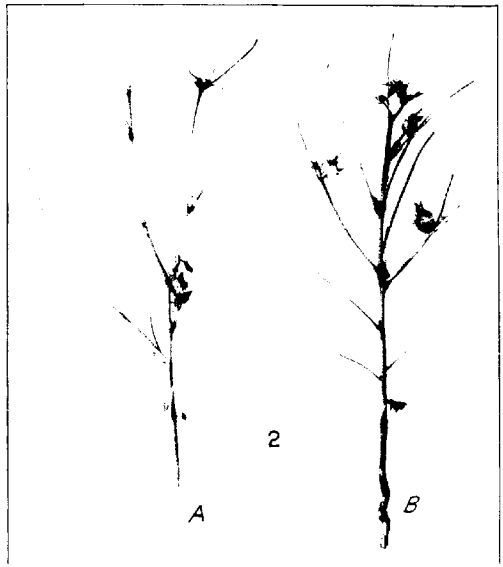
Fig. 1.—Underside of cotton leaf showing grasshopper injury. This shows a number of places where the very small nymphs ate only the epithelium and did not penetrate the leaf.

Fig. 2.—Cotton leaf showing grasshopper injury.

PLATE XVI

Fig. 1.—Injury to terminal bud of cotton by lepidopterous larva. This worm was embedded at point *a*.

Fig. 2.—Two cotton plants from laboratory garden with leaves removed. Plant *A* shows the abnormal forking caused by injury to the terminal bud, while *B* is a normal stalk. The absence of fruit on plant *A* is due to the deformity.


2
A
B

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